Whole genome sequencing for drug resistance profile prediction in Mycobacterium tuberculosis

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Abstract: Whole genome sequencing allows rapid detection of drug-resistant isolates. However, the availability of high-quality data linking quantitative phenotypic drug susceptibility testing (DST) and genomic data has thus far been limited.We determined drug resistance profiles of 176 genetically diverse clinical isolates from Democratic Republic of the Congo, Ivory Coast, Peru, Thailand and Switzerland by quantitative phenotypic DST for 11 antituberculous drugs using the BD BACTEC MGIT 960 system and 7H10 agar dilution to generate a cross-validated phenotypic DST readout. We compared DST results with predicted drug resistance profiles inferred by whole genome sequencing.Classification of strains by the two phenotypic DST methods into resistotype/wild type populations was concordant in 73-99 % of cases, depending on the drug. Our data suggests that the established critical concentration (5 mg/L) for ethambutol resistance (MGIT 960 system) is too high and may misclassify strains as susceptible, compared to 7H10 agar dilution. Increased minimal inhibitory concentrations were explained by mutations identified by whole genome sequencing. Using whole genome sequences, we were able to predict quantitative drug resistance levels for the majority of drug resistance mutations. Predicting quantitative levels of drug resistance by whole genome sequencing was partially limited due to incompletely understood drug resistance mechanisms. The overall sensitivity and specificity of whole genome-based DST were 86.8 % and 94.5 %, respectively.Despite some limitations, whole genome sequencing has the potential to infer resistance profiles without the need for time-consuming phenotypic methods.

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Title

Whole genome sequencing for drug resistance profile prediction in *Mycobacterium tuberculosis*

Authors

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Key words

Mycobacterium tuberculosis, quantitative phenotypic drug susceptibility testing, whole genome sequencing, drug resistance, drug resistance level prediction

Abstract

Whole genome sequencing allows rapid detection of drug-resistant *Mycobacterium tuberculosis* isolates. However, the availability of high-quality data linking quantitative phenotypic drug susceptibility testing (DST) and genomic data has thus far been limited.

We determined drug resistance profiles of 176 genetically diverse clinical *M. tuberculosis* isolates from Democratic Republic of the Congo, Ivory Coast, Peru, Thailand and Switzerland by quantitative phenotypic DST for 11 antituberculous drugs using the BD BACTEC MGIT 960 system and 7H10 agar dilution to generate a cross-validated phenotypic DST readout. We compared DST results with predicted drug resistance profiles inferred by whole genome sequencing.

Classification of strains by the two phenotypic DST methods into resistotype/wild type populations was concordant in 73-99 % of cases, depending on the drug. Our data suggests that the established critical concentration (5 mg/L) for ethambutol resistance (MGIT 960 system) is too high and may misclassify strains as susceptible, compared to 7H10 agar dilution. Increased minimal inhibitory concentrations were explained by mutations identified by whole genome sequencing. Using whole genome sequences, we were able to predict quantitative drug resistance levels for the majority of drug resistance mutations. Predicting quantitative levels of drug resistance by whole genome sequencing was partially limited due

to incompletely understood drug resistance mechanisms. The overall sensitivity and specificity of whole genome-based DST were 86.8 % and 94.5 %, respectively.

Despite some limitations, whole genome sequencing has the potential to infer resistance profiles without the need for time-consuming phenotypic methods.

Introduction

Timely and accurate drug susceptibility testing (DST) of *M. tuberculosis* isolates is vital to prevent the transmission of multidrug-resistant strains (MDR – resistance to rifampicin and isoniazid) (1).The slow growth and stringent biosafety requirements of *M. tuberculosis* make obtaining a full DST profile by culture-based techniques a matter of weeks or months. In addition, culture-based DST is notoriously challenging for several drugs, e.g. pyrazinamide and ethionamide due to poor drug solubility in commonly used culture media (2).

Drug resistance in *M. tuberculosis* is mainly conferred by chromosomal mutations in a few genes (3), making it possible to detect drug resistance by sequencing these genes or probing them by molecular hybridisation (4). Several commercial tests for the detection of resistance-associated mutations are available, e.g. the GenoType MTBDRplus V2 (Hain Lifescience GmbH, Nehren, DE) (5) and the AID TB Resistance Line Probe Assay (AID GmbH, Strassberg, DE) (6). The World Health Organisation (WHO) endorses line probe assays and the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) for the detection of rifampicin resistance as a surrogate marker for multidrug-resistance (7, 8). These molecular tests have high sensitivities for drugs with established target(s) of resistance and for which only a few mutations are responsible for most resistance *in clinico* (e.g. rifampicin, isoniazid) (4). However, these molecular tests show low sensitivity for heteroresistant strains (concomitant presence of wild type (wt) and mutant or multiple different resistant variants in patient isolates), when frequencies of mutant variants drop below 5-50 % (9, 10). Furthermore, there are no commercially available rapid tests for many drugs currently/prospectively in use (e.g. bedaquiline, delamanid, linezolid, p-aminosalicylic acid) and the WHO only recently defined *ad interim* critical concentrations for bedaquiline and delamanid for use with the BACTEC MGIT 960 system (11, 12).

A wealth of genomic data on drug-resistant *M. tuberculosis* has become available in the past years (13, 14). Unfortunately, quantitative phenotypic DST data are lacking for most of the genetic data sets, necessary to infer phenotypes from genotypes. In addition, DST data are

often limited as the strains were classified as susceptible or resistant using at the WHO-defined critical concentration (15). There is an urgent need to link genotypic and phenotypic drug resistance readouts to obtain a better understanding of the mechanisms influencing the evolution and spread of drug resistance in *M. tuberculosis* (3, 16).

WGS of clinical isolates allows for accurate identification of established chromosomal mutations increasing the minimal inhibitory concentration (MIC) (13, 17, 18) and may ensure adequate treatment in days instead of months. We compared whole genome-based drug resistance profiles with two culture-based quantitative DST methods for a total of 11 drugs, including rifampicin, rifabutin, isoniazid, all WHO group B (streptomycin, kanamycin A, amikacin and capreomycin), as well as selected group A (moxifloxacin), group C (ethionamide) and group D (ethambutol, pyrazinamide) drugs (11).

Material and methods

M. tuberculosis isolates

The initial data-set consisted of 189 *M. tuberculosis* isolates. A subset of 61 strains was used to establish the phenotypic DST methodology. These 61 strains were collected by the Swiss National Center for Mycobacteria between 2004-2015, and represent a broad spectrum in geographic origin and drug resistance profiles (19–21). We then applied the quantitative DST methodology to 125 prospectively collected clinical isolates from clinics participating in the International epidemiology Databases to Evaluate AIDS (IeDEA) (22) in Peru, Thailand, Ivory Coast and the Democratic Republic of the Congo (supplementary Table S3). Thirteen strains had to be excluded due to failed WGS (n = 4, failed library preparation due to low DNA quality), irreproducible DST results (n = 1), no growth in the 7H10 agar dilution assay (n = 3), duplication (n = 1), mixed cultures (n = 2, cross-contamination or patient infected with multiple strains) or transmission clusters (n = 2). The final set consisted of 176 strains.

Phenotypic DST

MGIT 960- and 7H10 agar dilution-based phenotypic DST were performed as described previously (19). Critical concentrations used for the classification of strains into resistant/susceptible aim to predict clinical outcome, i.e. treatment failure if a given strain is resistant at the critical concentration. However, critical concentrations should ideally be defined on the basis of the epidemiological cut-off (ECOFF: The highest wt MIC observed in

absence of any detectable resistance mechanism (23)), treatment outcomes and pharmako-kinetic and -dynamic data. As *M. tuberculosis* infections are treated with combination therapy, outcome data for single drugs are difficult to obtain (24). This calls for definition of critical concentrations solely based on the ECOFF (11). We therefore classified strains as belonging to the resistotype/wt populations on the basis the detection of growth/no growth at the ECOFF derived from our data (25). Table 1 lists the ECOFFs used, supplementary Table S2 the drug concentrations tested with the MGIT 960 and 7H10 agar-dilution assays and Table 2 the genes screened for mutations with WGS. Further details on how the phenotypic DST assays were performed are available in the supplementary materials.

Data analysis

The categorical agreement between classification of strains into resistotype/wt populations using MGIT 960 and 7H10 agar dilution was based on detectable growth at the ECOFF (Table 1). The numerical variation between the two methods was quantified as the geometric standard deviation (SD, given with its standard error) of the ratio MIC MGIT 960/MIC agar dilution, expressed as a number of 2-fold dilutions and denoted by σ. The geometric SD was computed by fitting a log-normal distribution to the ratio MIC MGIT 960/MIC agar dilution as implemented in the R package fitdistrplus (v.1.0-9) (26). If the data was compatible with σ = 0, the geometric standard deviation could not be estimated and was defined as "not applicable" (NA). The approach is a generalization of the Bland and Altman method (27), taking censoring of the data into account. Strains for which the MGIT 960 MIC and 7H10 agar dilution MIC were both left-censored or both right-censored were excluded since no information on the ratio could be derived.

Goodman and Kruskal's gamma was used to quantify the rank correlation between the two methods. No correlation could be calculated if the variance for either method was 0 and denoted with "not applicable" (NA).

Distributions of wt and mutant MICs were analysed qualitatively based on the results of 7H10 agar dilution. We divided the dataset into two groups: drugs for which the MIC distributions of wt and mutant strains did not overlap, and those for which MIC distributions overlapped.

Sensitivities and specificities of WGS-based resistance profile inference were calculated based on the 7H10 agar dilution results for all drugs, except pyrazinamide– for which the

MGIT 960 results were used, based on growth/no growth at the ECOFF, derived from our data and the presence or absence of a putative resistance-associated mutation.

Defining cut-offs for high/low-level MICs

The therapeutic window of a drug is defined as the concentration range within which a drug is considered to be effective and safe to use (28). Mutations can increase the MIC beyond the therapeutic window and render the drug clinically ineffective. Drugs may have large therapeutic windows beyond the ECOFF. For these, MIC increases caused by mutations may still be within the therapeutic window of a drug: these strains might still be treatable by increasing the drug dose. We analysed the distribution of MICs of mutant strains, and assessed if cut-offs for low-level (within the therapeutic window) and high-level (beyond the therapeutic window) MICs were definable. There were sufficient data available to define distinct cut-offs for low/high-level MICs for isoniazid, rifampicin, streptomycin and amikacin. For mutations conferring resistance to other drugs assayed in this study, no distinct separation into resistotype populations with high/low-level MICs was possible due to wide ranges of MICs conferred by the individual mutations or the mutations conferred MICs beyond the therapeutic window.

WGS and single nucleotide polymorphism (SNP) calling

WGS and data analysis was performed as previously described (29) and summarised in the supplementary materials. The performance of WGS-based DST greatly depends on the availability of robust markers of resistance. We therefore focussed on a set of high-confidence resistance-associated genes (3, 14, 28) (Table 2). We additionally assessed the impact of *eis* promotor mutations on amikacin and capreomycin resistance, as the association of mutations in the *eis* promotor with resistance to the aforementioned drugs has been reported but is not well established (11, 30).

Ethics

Local institutional review board or ethics committee approval was obtained at all local study sites. Informed consent was obtained where requested per local regulations. This project was also approved by the Cantonal Ethics Committee in Bern, Switzerland.

Results

Agreement between MGIT 960 and 7H10 agar dilution phenotypic DST

Table 3 and Figure 1 summarize the agreement between the semi-quantitative/quantitative MIC determination by MGIT 960 and 7H10 agar dilution in terms of classifying strains as belonging to the resistotype or wt populations as inferred by growth/no growth at the ECOFF (Table 1). Agreement was high for all drugs, except ethambutol (see below). For most drugs, the MGIT 960-based MICs were higher than the 7H10 agar dilution-based MICs. MICs obtained using the two methods were within 1-2 two-fold dilution steps of each other. The classifications into resistotype/wt populations demonstrated high rank correlations for most drugs (Table 3 and Figure 1), except for capreomycin (supplementary Figure S4) due to few strains demonstrating increased capreomycin MICs included in the study.

WGS and *in silico* resistance profile prediction

A total of 176 whole genome sequences with a median coverage of 676x (interquartile range [IQR] = 37.48) were obtained. Median mapping percentage and percentage of genome covered were 98.7 % (IQR = 0.94) and 99.4 % (IQR = 0.4), respectively. Genes involved in drug resistance demonstrated high coverages with only 0.8 % of all positions suffering from coverages below 7x (see supplementary materials). All major *M. tuberculosis* lineages, except lineage 7, were represented in the study (L1 = 6, L2 = 36, L3 = 11, L4 = 123, L5 = 1, L6 = 1). The strains showed a range of drug resistance profiles (Figure 2). Based on the set of analysed genes (Table 2), 25 strains were predicted to be fully susceptible against all assayed drugs, 59 strains were mono-/poly-resistant, 91 strains were MDR and two strains were predicted to be extensively drug-resistant (XDR: isoniazid, rifampicin, fluoroquinolone and aminoglycoside resistant).

Drug resistance profile prediction by WGS vs. phenotypic DST

After exclusion of known phylogenetic markers not involved in resistance, WGS-based resistotype prediction using a defined set of target genes (Table 2) was highly congruent with the categorical classification based on the phenotypic DST for most drugs (Table 3, Table 4, Figure 1). Based on the *in silico* resistotype prediction, the MICs of mutant and wt strains frequently followed a Gaussian distribution. Yet, the same resistance marker may confer different MICs in different strains (supplementary Figures S1C, S2C, S3C, S8C, S9C S10C). In some cases, the increase in the MIC conferred by a certain resistance mutation fell within

209 the distribution of the wt MIC (e.g. for *gidB*, *eis* promotor mutations, supplementary Figures
210 S3C S6C).

211 **Distinct wt and mutant MIC distributions**

212 MIC distributions of wt and mutant strains were well separated for rifampicin, rifabutin,
213 isoniazid, kanamycin A, amikacin, capreomycin, streptomycin and pyrazinamide, indicating
214 that the resistance markers used had a high positive predictive power (88.9 % overall positive
215 predictive power of associated with MIC increases). For streptomycin, two strains harboured
216 no mutations in the target genes, yet demonstrated high-level phenotypic resistance
217 (supplementary Figure S3C).

218 **Overlapping wt and mutant MIC distributions**

219 MIC distributions of wt and mutant strains overlapped for ethambutol, moxifloxacin and
220 ethionamide (Figure 3). For ethambutol and ethionamide, overlapping MIC distributions of
221 wt and mutant strains were associated with a large number of polymorphisms in resistance-
222 conferring genes (ethambutol resistance: 22 polymorphisms in *embB*, ethionamide resistance:
223 28 in *ethA*, 3 in *inhA*, 6 in *inhA* promoter). Solubility issues with ethionamide led to
224 quantitative differences in MGIT 960 vs. 7H10 agar dilution-based DST (Table 3, Figure 1).
225 The overlap in MIC distributions between wt and strains carrying an *embB* mutation was
226 reduced by adjusting the critical concentration for ethambutol resistance from 5 mg/L to 2.5
227 mg/L (MGIT 960). However, there was variability in the MICs for the same mutation (e.g.
228 MIC EmbB M306I/V in 7H10 agar dilution: 4-16 mg/L –supplementary Figure S2C).
229 Moxifloxacin resistance was rare (n = 9, MGIT 960, critical concentration 0.25 mg/L) and
230 MIC distributions of mutant strains partially overlapped with those of wt. Sensitivity of the
231 genome-based moxifloxacin resistance prediction was 80.0 % (Table 4).

232 **Defining cut-offs for high-/low-level MICs**

233 **Isoniazid**

234 Mutations in the promoter of *inhA* caused low-level MICs <1 mg/L (7H10 agar dilution),
235 compared to strains harbouring mutations in *katG* or combinations of *inhA* promoter and
236 *katG* mutations which demonstrated MIC levels ranging from >1 mg/L to >32 mg/L in 7H10
237 agar dilution (supplementary Figure S8C). Defining cut-offs for low- (≤1 mg/L for MGIT

960/7H10 agar dilution) and high-level (>1 mg/L MGIT 960/7H10 agar dilution) isoniazid MICs is warranted.

Rifampicin/Rifabutin

Most mutations in *rpoB* increased the MIC for rifamycins beyond the therapeutic window (peak serum concentration 10 mg/L (28, 31)). However, some rare *rpoB* mutations (e.g. RpoB L452P, H445L – supplementary Figure 9C) demonstrated MICs within the therapeutic window. Defining cut-offs for low- and high-level MICs may thus be justified.

For rifampicin, cut-offs for low-/high-level MICs were ≤4/2 mg/L for MGIT 960/7H10 agar dilution and >4/2 mg/L for MGIT 960/7H10 agar dilution, respectively.

For rifabutin, our data suggests a cut-off for low- and high-level MICs of ≤0.4/0.25 or 0.5 mg/L for MGIT 960/7H10 agar dilution and >0.4/0.25 or 0.5 mg/L for MGIT 960/7H10 agar dilution, respectively.

Mutations in *rpoB* conferring resistance to rifampicin and rifabutin showed highly correlated increases (Figure 4) of MICs beyond the therapeutic window for most *rpoB* mutations Figure 3 and supplementary Figure S9C & S10C), indicating that both drugs are rendered clinically ineffective by the mutations identified in the dataset (32) and cannot substitute each other.

Amikacin

Few strains had mutations in the regions of *rrs* relevant for amikacin resistance or the *eis* promoter (n=12). Mutations in *rrs* were associated with high-level (>128 mg/L in 7H10 agar dilution) MICs. With regards to the *eis* promotor, only the C-14T mutation increased the MIC and led to low-level (2-4 mg/L in 7H10 agar dilution) MICs. The definition of a cut-off for low- (≤ 4 mg/L for MGIT 960/7H10 agar dilution) and high-level (4 mg/L for MGIT 960/7H10 agar dilution) amikacin MICs may be warranted.

Streptomycin

Certain mutations lead to MICs well beyond the therapeutic window (28) of streptomycin (e.g. RpsL K43R, MIC 7H10 agar dilution >128 mg/L, supplementary Figure S3C). On the other hand, *gidB* mutations increase the MIC only moderately (MIC 7H10 agar dilution 1-4 mg/L, supplementary Figure 3C). Mutational combinations in *gidB*, *rrs*, *rpsL* were common and produced a range of different MICs. Despite the distribution of MICs conferred by

combinations of mutations, there were distinct mutations that systematically lead to MICs beyond the therapeutic window, e.g. RpsL K43R. Defining a cut-off for low-level (MGIT 960 ≤4 mg/L, 7H10 agar dilution ≤4-8 mg/L) and high-level streptomycin MICs (MGIT 960 >4 mg/L, 7H10 agar dilution >4-8 mg/L) is warranted.

Discussion

The results of MGIT 960 and 7H10 agar dilution-based phenotypic DST methods were highly correlated and suitable to separate the resistotype from the wt populations. Based on phenotypic DST results and WGS, we were able to define cut-offs for high- and low-level MICs for isoniazid, rifampicin, streptomycin and amikacin. Defining such cut-offs may serve as starting points for correlating mutational, DST and pharmaco-kinetic/dynamic data to gain more insight into the influence of individual mutations on treatment outcomes, especially in the light of e.g. increased drug dosing.

Our data suggest that the current WHO-defined critical concentration for phenotypic DST of ethambutol by MGIT 960 (5 mg/L) is too high and may misclassify strains as belonging to the wt population when compared to the 7H10 agar dilution-based classification. Given the narrow therapeutic window for ethambutol, this may lead to mistreatment due to presumed ethambutol susceptibility. After adjusting the ECOFF to 2.5 mg/L for MGIT 960, we observed a strong improvement of the categorical agreement between MGIT 960- and 7H10 agar dilution-based classification into resistotype/wt populations.

The mutations identified by WGS had a high predictive power to classify strains as belonging to the resistotype population. However, the predictive power depends on a number of factors. For instance, the increase in MIC conferred by an identical mutation can vary greatly in different strains (e.g. EmbB M306I/V, RpsL K88R) (33). Such variation may be clinically relevant if there is a significant overlap between the MICs of mutant and wt strains (23), as was the case for strains harbouring mutations in genes associated with ethionamide, ethambutol and streptomycin (e.g. *gidB*) resistance. . Furthermore, it is difficult to classify strains as part of resistotype or wt populations if the MIC increase lies within the therapeutic window of a drug. The overlap between MICs of mutant and wt strains is confounded by the fact that we only screened for mutations in genes which had previously been associated with drug resistance. We might thus have missed possible resistance-conferring mutations in other genes. Additionally, WGS will always produce distributions of coverages, which in term will

298 inevitably lead to certain regions in the genome suffering from low coverage, preventing the
299 detection of mutations. However, in cases where we observed elevated MICs without any
300 mutations detected the target genes, coverage issues could not explain the absence of any
301 mutations. Furthermore, the strain genetic background (34), non-mutational mechanisms (e.g.
302 modulation of gene expression) (35), as well as drug efflux mechanisms (36) may contribute
303 to the variability in increase of the MIC conferred by resistance mutations.

304 The predictive power of mutations in target genes also depends on removing phylogenetic
305 markers not involved in increasing MICs. Separating phylogenetic from resistance-associated
306 markers works well for essential (highly conserved) genes such as *rpoB*, *rpsL, rrs* but is
307 problematic in non-essential genes involved in the conversion of prodrugs into their active
308 forms like *pncA* (pyrazinamide), *ethA* (ethionamide) or in genes that generally exhibit higher
309 numbers of polymorphisms e.g. *embB*. Of note, the *embABC* operon is highly polymorphic,
310 harbouring more polymorphisms than expected by chance (mutations in *embABC* operon =
311 81, expected = 44.8, p = 9.174e-07, binomial test). Mutations conferring increased ethambutol
312 MICs (37) will therefore inevitably evolve in the presence of phylogenetic SNPs and may
313 interact epistatically to produce the variability in MICs we observed for wt strains and for the
314 most common marker associated with increased ethambutol resistance MICs, *embB*
315 M306I/V. The *embABC* operon is involved in the biosynthesis of decaprenylphosphoryl-β-d-
316 arabinose, which is an integral component of the mycobacterial cell wall. The cell envelope
317 interacts with the host immune system and the high levels of diversity of these genes might
318 be the product of diversifying selection due to host immune pressure. The influence of
319 polymorphisms in the *embABC* operon on MICs in general is supported by the observation
320 that sub-inhibitory concentrations of ethambutol lower the MICs for isoniazid, rifampicin and
321 streptomycin (38). Even small changes in activity of the decaprenylphosphoryl-β-d-arabinose
322 biosynthetic and utilisation pathway might thus alter cell wall permeability and influence
323 MICs of several drugs.

324 Similarly, in the case of increased streptomycin MICs, the RpsL substitution K88R exhibited
325 a wide range of MIC increases, partially within the therapeutic window of the drug.
326 Streptomycin was the first effective antituberculous drug discovered (39) and has been used
327 for decades. The long-term use has produced complex resistance profiles with multiple
328 mutations known to increase streptomycin MICs on their own (e.g. in *gidB*, *rpsL, rrs*)
329 occurring concomitantly, producing wide ranges of MICs. Furthermore, many strains with
330 increased streptomycin MICs displayed MDR/XDR phenotypes. Mutations conferring

increased streptomycin MICs are frequently found in backgrounds which have mutations in genes affecting the information pathway (DNA -> RNA -> proteins) – e.g. *gyrA* (DNA gyrase), *rpoB* (DNA-dependent RNA polymerase), *rrs* (ribosomal RNA). The simultaneous presence of multiple MIC increasing mutations may alter the adaptive landscape (40, 41). In addition, non-mutational processes (e.g. alteration of gene expression) may compensate for fitness costs of drug resistance and at the same time alter the MIC for the drug (35). This has not been demonstrated for streptomycin resistance in *M. tuberculosis*, but it seems possible that compensation of fitness costs in MDR phenotypes might alter the MIC for streptomycin (40), considering that streptomycin is not part of the current standard treatment regimen and selection for high-level streptomycin MICs is relaxed.

Concerning *eis* promoter mutations and aminoglycoside resistance, there is mounting evidence that the *eis* C-14T promotor mutation may confer clinically relevant increases in amikacin MICs, especially in the light of the revised critical concentrations for amikacin (2 mg/L, 7H10 agar dilution) [11].

We observed an overrepresentation of lineage 4 and 2 strains in our sample set. The strain set used to establish the methodology was collated with a specific aim to include drug-resistant strains and given the frequent association of lineage 2 and 4 with drug-resistance (42, 43), the observed skew is not surprising. Furthermore, lineage 2 and 4 strains are also frequently isolated at the collection sites of the strain set used to apply the methodology (Ivory Coast, Peru and Democratic Republic of the Congo). Similarly, increased MICs for a number of drugs (amikacin, capreomycin, kanamycin, moxifloxacin) was rare, reflecting the scarcity of pre-XDR/XDR phenotypes in Switzerland and at the sites of prospective sampling.

With 63.6 % – 80.8 %, sensitivities were low (44) for a number of drugs (i.e. for amikacin, moxifloxacin, pyrazinamide) (table 4), but were comparable to other studies not employing a database of pre-defined resistance mutations (14, 17, 45). The observed low sensitivities for some drugs were either due to few strains belonging to the resistotype population included in the dataset, the presence of additional resistance mutations in genes not assessed or due to unknown resistance mechanisms and not due to low coverages prohibiting the detection of mutations. The use of a curated SNP-database containing high-confidence drug-resistance mutations would improve sensitivity for some drugs where additional targets, less well associated with MIC increases, are known (44, 46). However, reliance on a predefined resistance mutation database comes at the cost of reduced sensitivity. After known

phylogenetic mutations have been removed, it is important to treat any mutation in known target genes as potentially involved in drug resistance. In cases where previously unknown mutations (i.e. neither known to increase MICs, nor a known phylogenetic SNP) in resistance-related genes are detected, genetic engineering and targeted DST is necessary to confirm or reject the drug-resistance conferring nature of a novel mutation to achieve high sensitivities and specificities for whole genome sequencing-based DST.

Generating high-quality quantitative DST data using diverse *M. tuberculosis* strains is important to accurately define the ECOFF and subsequently guide treatment decisions. The two quantitative DST methods employed are difficult to standardize across laboratories, technically demanding and at best challenging to scale up. Microtiter plate-based quantitative DST methods (47, 48) have the potential to aid in the generation of more high quality DST data due to their standardized formulation and relative ease of application compared to established methods.

In conclusion, we demonstrate that MGIT 960 and 7H10 agar dilution-based phenotypic DST provide highly congruent classifications of strains into resistotype or wt populations. WGS has high predictive power to infer resistance profiles without the need for time-consuming phenotypic methods. Limitations due to overlapping distributions of wt and resistotype MICs, varying MICs for the same mutations in different strains, presence of phylogenetic markers in resistance-associated genes and rare resistance markers with low frequencies will likely be resolved by on-going large-scale projects (e.g. ReSeqTB and others (15, 49)) combining phenotypic DST with WGS of thousands of *M. tuberculosis* isolates. Our findings, together with those of on-going studies will pave the way for the replacement of phenotypic DST with drug resistance profile prediction based on WGS in the coming years.

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401 ## Conflict of interest

402 Peter M. Keller reports travel grants by Copan Italia SpA outside of the submitted work. Erik
403 C. Böttger is a consultant for AID Diagnostics.

404

Tables

Table 1 Epidemiological cut-offs (ECOFF) used for 7H10 agar dilution and MGIT 960 phenotypic DST, derived from wild-type MIC distributions determined in this study. The values given in parentheses are the critical concentrations recommended by the WHO in 2014 [22].

Antibiotic	ECOFF agar dilution (mg/L)	ECOFF MGIT 960 (mg/L)
Ethionamide	1 (5)	5
Ethambutol	2 (5)	5
Capreomycin	4	2.5
Streptomycin	0.5 (2)	1
Kanamycin A	2 (5)	2 (2.5)
Amikacin	1 (4)	1
Moxifloxacin	0.25 (0.5)	0.25 (0.5)
Isoniazid	0.125 (0.2)	0.1
Rifampicin	0.5 (1)	1
Rifabutin	0.0625	0.1
Pyrazinamide	NA	100

Table 2 List of genes implicated in drug resistance in *M. tuberculosis* which were screened for polymorphisms by WGS. List adapted from [3, 12, 23].

Drug	Target gene(s)
Ethionamide	*ethA, inhA, inhA* promoter
Ethambutol	*embB*
Capreomycin	*rrs, eis* promoter, *tlyA*
Streptomycin	*rrs, gidB, rpsL*
Kanamycin A	*rrs, eis* promoter
Amikacin	*rrs, eis* promoter
Moxifloxacin	*gyrA*
Isoniazid	*katG, inhA* promoter
Rifampicin/rifabutin	*rpoB*
Pyrazinamide	*pncA, pncA* promoter

Table 3 Summary statistics of the method agreement between 7H10 agar dilution- and MGIT 960-based phenotypic DST for all drugs assayed in this study.

Antibiotic	n	Categorical agreement (%)	SD of $\log_2$(MIC MGIT 960/MIC agar dilution)	γ
Ethionamide	56	95	1.9 ± 0.3	0.91
Ethambutol	171	73	1.9 ± 0.5	0.94
Capreomycin	56	98	1.5 ± 0.5	0.65
Streptomycin	56	93	1.5 ± 0.3	0.98
Kanamycin A	56	98	1.2 ± 0.2	0.8
Amikacin	174	98	1.4 ± 0.6	1
Moxifloxacin	173	99	1 ± 0.2	1
Isoniazid	173	96	1.2 ± 0.1	1
Rifampicin	174	99	NA	1
Rifabutin	56	96	0.8 ± 0.1	0.98

Table 4 Sensitivity and specificity of the genome-based drug resistance profile prediction using the 7H10 agar dilution-based categorical classification as the gold standard for all drugs except pyrazinamide, for which the MGIT 960 categorical classification was used.

Drug	Sensitivity (%)	Specificity (%)
Ethionamide	75.0	92.9
Ethambutol	89.6	94.2
Capreomycin	75.0	94
Streptomycin	68.0	92.1
Kanamycin A	83.3	98.8
Amikacin	63.6	96.9
Moxifloxacin	80.0	90.2
Isoniazid	93.6	96.8
Rifampicin	100	94.0
Rifabutin	98.9	94.0
Pyrazinamide	80.8	88.9

Figure legends

Figure 1
Method agreement between phenotypic DST performed with MGIT 960 and 7H10 agar dilution represented as Bland-Altman plots for all drugs tested in this study.

Figure 2
Maximum likelihood phylogeny of 176 *M. tuberculosis* strains based on 20510 variable positions. Reference strains labeled with green tip labels. Main lineages are highlighted as follows: red L4, purple L3, blue L2, pink L1, green L6, brown L5. Scale bar indicates number of substitutions per site. Phylogeny rooted on *M. canettii*. Colored bars indicate resistance

mutations per gene and within a distinct column (gene) each colored bar represents a distinct mutation. Black bars indicate no mutation, i.e. wt.

Figure 3

Histograms of MICs (7H10 agar dilution) for all drugs assayed in this study

Figure 4

Correlation between 7H10 agar dilution MICs for rifampicin and rifabutin

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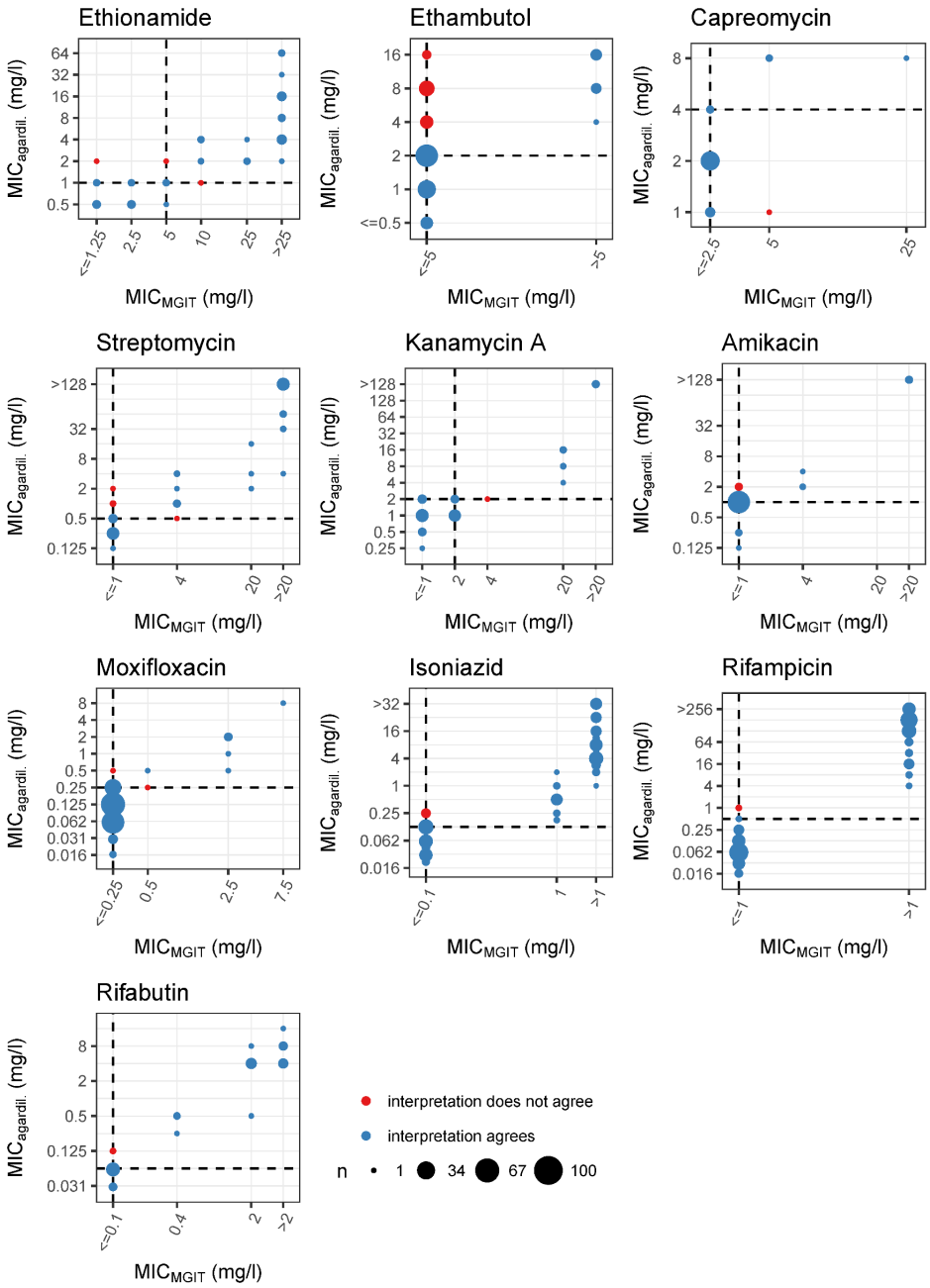
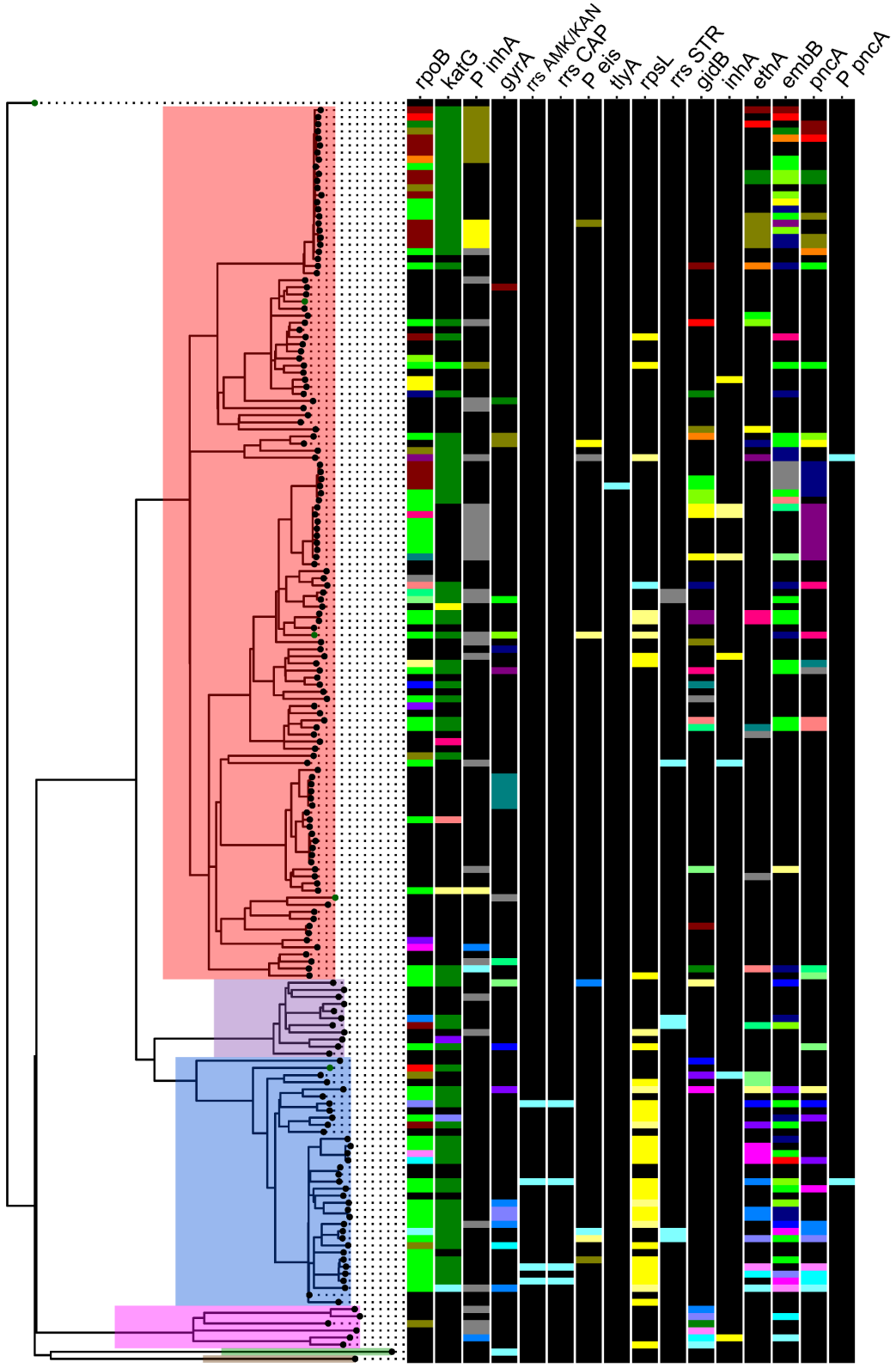


Figure 1 Method agreement between phenotypic DST performed with MGIT 960 and 7H10 agar dilution represented as Bland-Altman plots for all drugs tested in this study.



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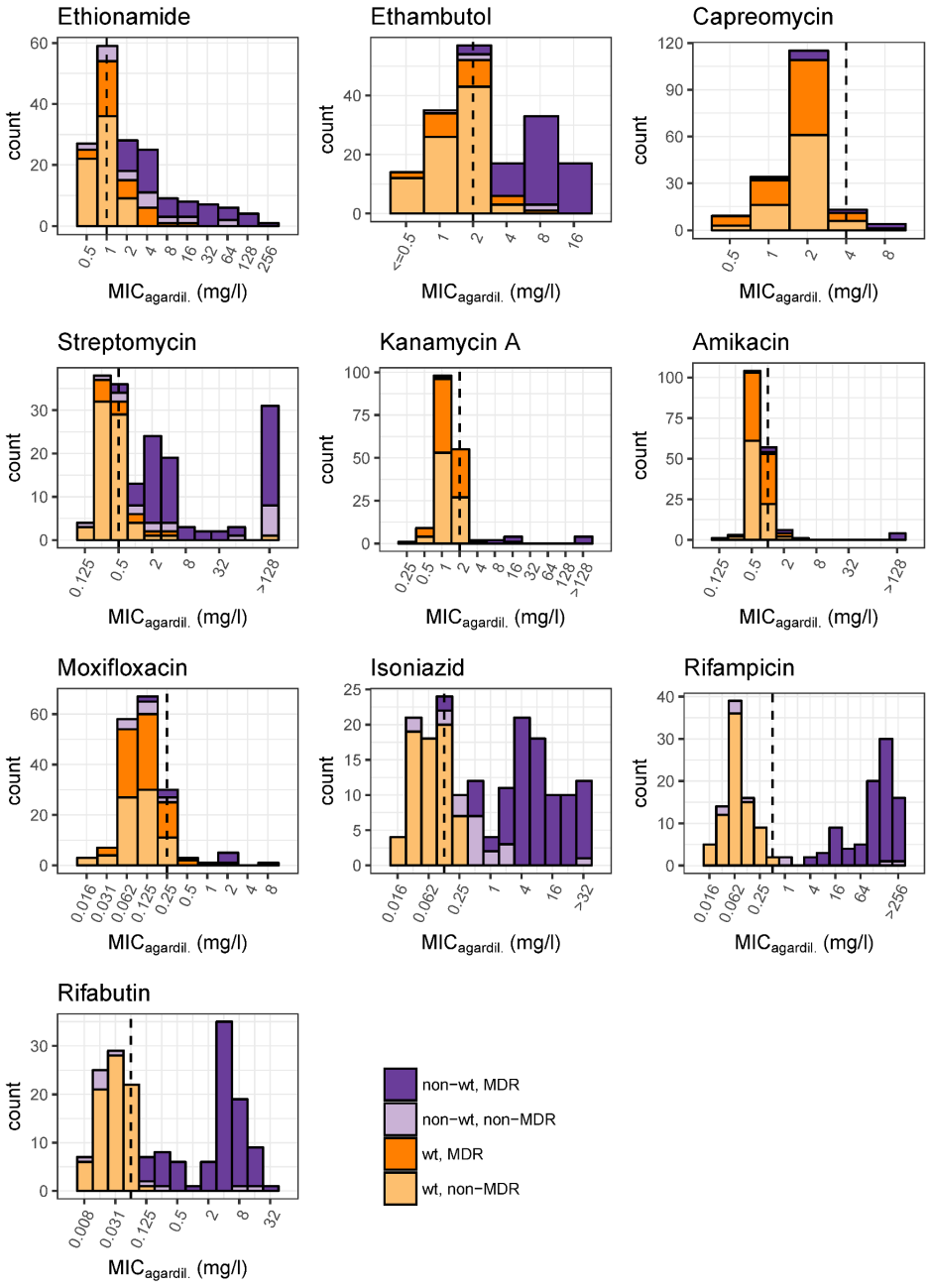

Figure 3 Histograms of MICs (7H10 agar dilution) for all drugs assayed in this study

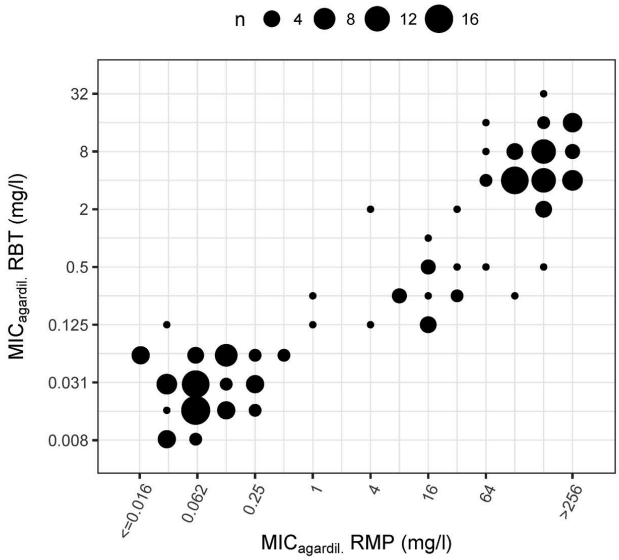

Figure 4 Correlation between 7H10 agar dilution MICs for rifampicin and rifabutin

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